a1 corrd.

continuation-in-part of PCT/JP95/02035, filed 02 October 1995, now U.S. Patent No. 6,110,708--

Please delete the paragraph at page 2, lines 19-25, and substitute the following paragraph:

a2

Similar methods had also been tried by using another expression vectors, but the same or less expression level had merely detected by any of the vectors. Anyway, an effective expression system have not yet been realized in the art. This seems due to difficulties in expressing the conglutinin because *Escherichia coli* does not possess proteins of the structure like collagen-like region. Further, yield of the conglutinin produced from an eukaryotic cells is little, and some of the conglutinin may sometimes have an inappropriate post-transcriptional modification.

Please delete the paragraph at page 4, lines 9-10, and substitute the following paragraph:

a3

Figure 8 shows conglutination activities on the recombinant conglutinin and the native conglutinin with microtiter plate assay system;

Please delete the paragraph at page 5, lines 21-25, and substitute the following paragraph:

a4

PCR products of Example 1(1) were digested with the restriction enzymes XhoI and EcoRI, then were inserted into the expression vector pRSET-A (Invitrogen) with DNA ligation kit (Takara Shuzo). Then, pRSET vector so prepared from pRSET-A containing bovine conglutinin cDNA fragment was transfected into *Escherichia coli* JM109 and transformants were obtained that have the conglutinin DNA fragments corresponding to 631 bp through 1113 bp of the native conglutinin DNA (Figure 1).

Please delete the paragraph at page 5, line 26, to page 6, line 2, and substitute the following paragraph:

a5

Sequences of these fragments were corresponding to 191st through 351st amino acids of native conglutinin, namely, PCR exactly amplified the sequences having the short collagen region, the neck region and the carbohydrate recognition domain. Further, there was

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no error in the PCR reaction. Accordingly, desirable stable transformants were obtained which can remarkably produce such conglutinin DNA fragments.

Please delete the paragraph at page 10, lines 1-9, and substitute the following paragraph:

a6

After coating the microtiter plates with yeast mannan (1 μg/well), the recombinant conglutinins were reacted with sugars. Sugar binding specificity (I_{50}) was shown as sugar concentration to halve binding activities. Results are shown in Table 1. Obviously from Table 1, sugar binding activities with the recombinant conglutinin are substantially same to that of the native conglutinin. Then, as shown in Figures 6 and 7, like the native conglutinin, binding activities of the recombinant conglutinin were dependent on calcium ion. Further, these binding activities were inhibited by N-acetylglucosamine. On the other hand, tags of histidine fused to the recombinant conglutinin were not involved in the binding activities to mannan and binding specificities.

Please delete the paragraph at page 11, lines 25-30, and substitute the following paragraph:

a7

Conglutination activities of the recombinant conglutinin and the native conglutinin were evaluated by Microtiter plate assay system. Sheep erythrocyte cells with iC3b were prepared according to the method of Wakamiya *et al.*, (*Biochem. Biophys. Res. Comm.*, Vol. 187, pp. 1270-1278, 1992). Namely, 1 % sheep erythrocyte cells with iC3b were prepared by priming with a mixture of ten-fold diluted fresh horse serum and equivalent amount of anti-Forssmann antibody, and incubated at 37 °C for ten minutes.

Please delete the paragraph at page 12, lines 1-10, and substitute the following paragraph:

a8

50 μl of 1 % sheep erythrocyte cells with iC3b and 50 μl of the recombinant conglutinin or 50 μl of the native conglutinin were added to the raw veronal buffer or the veronal buffer containing 30 mM N-acetylglucosamine. Then, they were incubated at 37 °C and the conglutination activities thereon were detected. The lowest concentration of the proteins to cause agglutination is regarded as titer of conglutination, then the results are shown in Figure 8. In Figure 8, Lane A is the native conglutinin, Lane B is the recombinant

conglutinin and Lane C is the recombinant conglutinin containing 30 mM N-acetylglucosamine. Titer of conglutination on the native conglutinin was 0.16 μg/ml, while that of the recombinant conglutinin was 1.3-2.5 μg/ml. Such activities were completely inhibited by 30 mM N-acetylglucosamine (GlcNAc).

Please delete the paragraph at page 12, line 22, to page 13, line 4, and substitute the following paragraph:

In accordance with the method of Okuno *et al.*, (*J. Clin. Microbiol.*, Vol. 28, pp. 1308-1313, 1990), experiments were performed in 96-well microtiter plates with 1 % chick's erythrocytes. The ether-treated virus antigens from a hen egg antigen was used. No additive had been added to mixed cultivation solution of TBS/C (TBS solution containing 5 mM sodium chloride) except for 30 mM N-acetylglucosamine or 10 mM EDTA. After incubation at room temperature for one hour, effects on the recombinant conglutinin fragments (rBKg-CRD) against viral hemagglutination on chick's erythrocytes were observed. Results are shown in Table 2. Results on Influenza A virus A/Ibaraki/1/90 are shown in Figure 9. In Figure 9, Lane A is the native conglutinin, and Lanes B, C and D are directed to the recombinant conglutinin fragments, in which the Lane B is no additives, Lane C is added thereto 30 mM N-acetylglucosamine and Lane D is added threreto 10 mM EDTA.

Please delete the paragraph at page 13, lines 18-22, and substitute the following paragraph:

Hemagglutination Inhibition (HI) activities were depended on dosages and calcium. Further, Hemagglutination Inhibition (HI) activities of the recombinant conglutinin are substantially the same level to the titer of the native conglutinin, rat surfactant protein D, human surfactant protein D (Hartshorn *et al.*, *J. Clin. Invest.*, Vol. 94, pp. 311-319, 1994).

Please delete the paragraph at page 15, lines 19-25, and substitute the following paragraph:

Physiological activities against Influenza A viruses were evaluated in accordance with the evaluation method on Hemagglutination Inhibition (HI) Activities according to Example 5, the evaluation method on Neutralization Activities according to